

JL

Melbourne
October 3, 1957

Dear Joe: *lein*

Thank you for the shipping labels for the soil samples. I've sent someone to Roy Edmonds in Fiji, and will do the same for Hawaii and Australia. If we go through India, I'll look out for possible contacts there.

We've been settled in here at Melbourne for just about a month, and now have to look ahead to our further travel plans. We'll be in Melbourne till the end of this month, may take November to return by a flight plan: Adelaide-Perth-Calcutta-Delhi-Bombay-Karachi-Athens-Rome-Milan-London and home. We have no thought (time or energy) to stopover except a ~~sample~~ fortnight in India and a week (with Cavalli) in Milan. This is not yet certain, but in any case any correspondence that would arrive after Nov. 1 should be routed to Madison.

I hope you can plan to visit us in Madison some time during the next year, so we can show off some of our kodachromes. But to tell the truth, it has been rather depressing what a small fraction give as spectacular a picture as one saw through the sighting lens.

The lecture course has been going rather smoothly, though Esther has had the short end of the stick, having to manage a rather frantic practical (lab) course with well-meaning but not very well equipped assistance. I've been mainly preoccupied with my work in Burnet's lab, and this has given me a very rewarding glimpse of the methodology of work with animal viruses. My first experiments on the nature of incomplete virus have not been very rewarding, but by happenstance they led to some possibly useful observations on better selective systems for the analysis of recombination, which is still in a rather primitive stage except for the certainty of its existence in influenza. These have also provoked the following reflections on the possibility of antiviral chemotherapy.

First of all, I would still take issue with Amel's pessimism on the value of such chemotherapy. Even for acute respiratory infections, there is certainly an interval of several hours to a day or two between prodromal symptoms and the acute attack. Furthermore, in an epidemic situation like that for Asian Flu, a prophylactic agent, or one that mitigated an initial infection, would be extremely useful. This strain of flu does not seem to be a very effective antigen, and I will be interested to see if any evidence comes up that vaccination has any significant effect. The Australian episode came (and has now went) too rapidly for an accurate assessment, and the same may be happening now in the US. The Sydney newspapers, especially, were quite exercised about what they called the 'golden death', viz. staphylococcal (aureus) ~~inf~~ pneumonias, of which there have been a few cases during the epidemic.

Of the various aspects of viral growth, it does not seem to me that we have enough fundamental information on any of the intracellular

processes for any intelligent planning of chemotherapeutic research. we might take encouragement from the fact that infected cells contain no significant content of infective (flu) virus: i.e. that mature virus is liberated as soon as it formed, or that maturation entails the egress of the virus particle. The latter is not implausible, on the notion that the emerging virus particle picks up its skin from the host cell. But there is still no clear direction for the means to modify the surface of the host cell so as to prevent normal maturation. (This is all tied in with the problem of 'incomplete virus').

Much more is known about the earliest stages of virus penetration, and the biochemical specificities of the free virus. The most clearcut of these, for flu, is the 'attachment effector' usually measured as the hemagglutinin activity of the virus. As you know, the specific receptors for this attachment have been identified as mucoproteins, of which the prosthetic group is neuraminic acid (Gottschalk has a useful review on this in *Physiol. Reviews* last year). (The structure of neuraminic acid has a remarkable resemblance to that for muramic acid of bacterial walls.) It is also true that the effector (hemagglutinin=HA) has an enzymatic action (neuraminidase) which destroys the effector sites, viz. in the elution of virus from HA'd rbc. The view of the Australian virologists is that this enzymatic activity is not essential for penetration, though it may play some role in releasing virus conjugated with soluble receptor molecules (assayed as inhibitors of HA) and also releasing virus on its way out where it may have to chew through considerable mucoid.

While the enzymatic activity is dispensable, it is reasonably clear that the attachment of HA to receptor sites is a necessary preliminary to the uptake of the virus. Infectivity can thus be greatly reduced or delayed by the presence of excess soluble receptors; however, for some reason this effect has not been stressed. (My own selective agents are, of course, various mucoids; it is true that their effect on infectivity is limited, and by itself not so striking or useful as that of antibody, but it is quite real.)

The point is that the neuraminidase activity of the virus limits the inhibitory effect of the natural substrates. It is already well known that the periodate-treatment of various mucoids, at appropriate levels, will make them unsplitable without preventing them from binding with the HA. There have been some desultory trials of periodated mucoids as curative agents; I haven't read all the literature as yet, but I doubt if the issue is entirely closed. What might be most useful would be a similar agent of low molecular weight, so that it would be ready diffusible, which had similar properties. And of course, I am going to propose your search for such an agent, either by the well established random methods, or by 'substitutive chemotherapy'. As far as I can tell there has been no concerted effort whatsoever to modify HA-reactive mucoproteins with other chemical reagents. Neuramin-lactose has been synthesized by Gottschalk, and is the simplest substrate of neuraminidase; it does not even inhibit hemagglutination, presumably because of rapid enzymatic degradation. It, and similar compounds, have also been isolated from natural sources, and in the event of a promising substitution result would be plausible starting points for a low mw

inhibitory product.

What I don't know is the extent of your present interest and facilities in virus work. Many of these remarks may be supererogatory to you, but it seems best to start from scratch.

There is little doubt that other companies have looked for natural products which might inhibit hemagglutination, this being the simplest possible test for their effector-blocking activity, but this is not necessarily a valid argument against your doing the same. The test is an extremely simple one-- in fact, if you can get someone else to ship you the infected egg fluids, you don't have to grow the virus yourself at this level. Further, the egg-adapted strains of flu are almost certainly of negligible pathogenicity for man. The more interesting features would be tests of artificially substituted materials, which are not too difficult to get hold of or prepare: e.g. sheep salivary-gland mucin is one of the most effective. The egg techniques are not difficult at all, and if they are not already involved, any of your microbiologists could pick them up.

A second point that intrigued me a great deal was a simplified method (at least a cheaper one) of titration of infectivity that Fazekas has developed at Canberra. I'll send you a copy when I get home, but briefly he just prepares a de-embryonated egg (that is an 11-14 day old egg emptied of all its contents, and left only with the single layer of parietal chorio-allantoic membrane.) These DEE's have been widely used already as convenient test-tubes for growing flu, since they can be washed out, fluids sampled and replaced, etc. Fazekas goes one step further, and cuts the shell, with adherent membrane, into squares about 6mm wide, places each square in a separate cup in an HA titrating tray (a large spot plate molded from plastic) with .3 ml of glucose-chloramphenicol-gelatin-Ringers, and titrates out the virus on these bits. (Each shell furnishes about 75 bits.) After 48 hours incubation, with shaking, the trays are read by picking out the bits, adding a drop of fowl rbc and counting the fraction of cups with hemagglutinin (i.e. virus) at the various dilutions. We have been repeating the technique here, and it looks quite promising.

Well, I've rambled on enough. I know that you have more 'promising approaches' to consider, than means to pursue them, but I do think that the improvement of methods makes a new ~~consideration~~ appraisal of what is worth while doing in your own context very desirable. As usual, do let me know your views.

Yours,

Joshua Lederberg